

# AN ANGLE SENSE IN THE ORIENTATION OF A MILLIPEDE<sup>1</sup>

FRANKLIN H. BARNWELL

*Department of Biological Sciences, Northwestern University, Evanston, Illinois,  
and the Museu Paraense "Emilio Goeldi," Belém-Pará, Brazil*

The literature of experimental psychology contains numerous studies of a maze-running phenomenon which has been called "reverse turning." This term refers to the subsequent reaction of an animal which has been forced to make a right-angle turn in a maze immediately before encountering a free choice point where it can turn to right or left. At the choice point an animal exhibiting reverse turning, turns to the side opposite that of the preceding forced turn; for instance, a forced left turn is followed by choice of a right turn. This alternation of turning direction has been reported for a variety of organisms throughout the animal kingdom. The reasons for studying the phenomenon have been diverse and have led to several different interpretations. It seems worthwhile, since it has not been done before, to review the different approaches to the problem.

The first extensive examination of reverse turning in a maze was made by Schneirla (1929) in his studies of learning in ants. He found a strong tendency for two species of *Formica* to alternate turning directions at a choice point immediately following a forced right-angle turn. Schneirla was interested in the phenomenon from the standpoint of maze design for the study of learning. The choice at a junction within the maze could be strongly influenced by the pattern of the preceding turns. If the reverse turning tendency were operating and the choice it favored were a blind alley, turning into this alley would be eliminated only with great difficulty during learning of the correct maze pathway. Schneirla explained reverse turning by "centrifugal swing," in which he distinguished two components, the first being the effect of momentum and the second, a thigmotactic response. According to his interpretation, the running ant, upon entering a right-angle turn, would be carried by its momentum into contact with the outside wall of the turn; subsequently the ant would follow this wall and, at the free choice point, would tend to turn toward the side of antennal contact.

Soon afterward, reverse turning was reported in the maze running of white rats by Dashiell and Bayroff (1931). During maze running in rats, these authors did not observe any obvious bodily displacements which corresponded to those upon which the centrifugal swing theory was based. To account for the reverse turning they proposed that "the factor most responsible is a forward-going tendency in animal locomotion that leads not only to maintenance for short distances of a direction already set but also to a compensatory sort of correction when forced out of line by an obstruction" (p. 94).

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Following the report of Dashiell and Bayroff (1931) there appeared a number of papers (Schneirla, 1933; Ballachey and Buell, 1934; Witkin and Schneirla, 1937) which attempted to demonstrate that the reverse turning of rats could be accounted for by the centrifugal swing principle and that it was unnecessary to invoke any innate "forward-going tendency." Apparently these arguments for centrifugal swing were not wholly convincing. While acknowledging the important role of mechanical inertia and displacement in maze performance, Warden, Jenkins and Warner (1940, p. 805) wrote, "A more likely interpretation of the 'reverse turn' subsequently displayed at the junction, however, is that the phenomenon is primarily the *direct* outcome of behavioral inertia (postural activity) based in part on stimulation of tension receptors through inertial changes precipitated at the previous turn and in part on the set of the organism antecedent to the previous turn."

In 1948 Hullo observed reverse turning in the cockroach *Blattella germanica* run on an elevated maze. The turning was attributed to centrifugal swing.

In the 1950s there was a renewal of interest in reverse turning. Demonstrations of its occurrence in several organisms were presented as evidence for Hull's principle of reactive inhibition. Hull stated the principle as follows (1943, p. 300): "Whenever a reaction is evoked in an organism there is created as a result a primary negative drive; this has an innate capacity to inhibit the reaction potentiality to that response; the amount of net inhibition generated by a sequence of reaction evocations is a simple linear increasing function of the number of evocations; and it is a positively accelerated increasing function of the work involved in the execution of the response; reactive inhibition spontaneously dissipates as a simple negative growth function of time." Presumably, then, in the maze situation there would occur, following a forced turn to the right, a temporary inhibition of the right-turning tendency, and at the next choice point the animal would turn left. As support for this concept Lepley and Rice (1952) reported that *Paramecium multimicronucleatum* in a microscopic maze tended to alternate its turning direction following a forced turn. However, Jensen (1959) pointed out that centrifugal swing had not been controlled in these experiments, and that this latter alternative seemed the more plausible explanation. Furthermore, using *Paramecium caudatum*, Lachman and Havlena (1962) were unable to reproduce the results of Lepley and Rice. Perhaps it should be noted that this experiment differed from the experiment of Lepley and Rice in that the choice occurred at a Y rather than a T junction. This point is mentioned because it has been suggested that "invertebrates" may not alternate turning directions at a choice point in a Y-maze (Hayes and Warren, 1963). Apparently this generalization was based on the report that the beetle, *Aleochara bilineata*, did not exhibit spontaneous alternation (see below) in a Y-maze (Putnam, 1962). Even in this case, however, turning of the beetle in the maze was not random. Instead of alternating, the beetle showed a highly significant tendency to repeat its preceding choice.

Mealworms, larvae of *Tenebrio molitor*, were found to alternate turning directions after a forced turn (Grosslight and Ticknor, 1953). The tendency to alternate was greater following two consecutive turns in the same direction and decreased when the distance between forced turn and choice point was increased. Following Jensen's (1959) criticism that centrifugal swing, in particular the

thigmotactic component, had not been controlled, the experiment was repeated with a narrow maze in which the mealworm was presumably equally stimulated on both sides of its body at the choice point (Grosslight and Harrison, 1961). The authors found that even with thigmotaxis controlled, the reverse turning tendency persisted with the same intensity found in the earlier study. They interpreted their results in terms of reactive inhibition.

Dingle (1964b) has confirmed the occurrence of reverse turning in *Tenebrio* larvae, but he has made additional observations which he believes are incompatible with the principles of reactive inhibition, as originally formulated by Hull (1943). First, using a maze which presented the three alternatives of turning right or left or continuing straight ahead, he found that following a forced turn to the right, no animals chose to turn right and the number turning left was slightly greater than the number continuing straight ahead. Control animals not subjected to forced turning chose to continue straight ahead. Thus, the strong tendency for left turning following forced right turning reflected not simply inhibition of right turning, but rather a tendency to counteract specifically the preceding forced right turn. Second, reverse turning increased as the distance between starting point and forced turn was increased. Thus, the intensity of reverse turning in *Tenebrio* was dependent upon events occurring prior to the forced turn. Third, when the distance between forced turn and choice point was increased from four to eight centimeters, reverse turning disappeared and most larvae chose to continue straight ahead. However, when larvae were retained under cotton wool immediately following the forced turn, for a period of time necessary to crawl eight centimeters, and then were permitted to crawl four centimeters to the choice point, they evinced strong reverse turning. Thus, within the limits studied the decay of reverse turning tendency was not a function of time but rather of distance crawled. For these reasons Dingle rejects reactive inhibition as an explanation of reverse turning in *Tenebrio*.

The terrestrial isopod, *Armadillidium vulgare*, was reported to alternate its turning directions with high significance at successive choice points in a maze (Watanabe and Iwata, 1956). Reverse turning tendency was greater following two consecutive turns toward the same side and decreased as distance between the choice point and preceding turn increased. Results were interpreted in terms of reactive inhibition. The authors did not consider the possibility that their results could be explained by centrifugal swing.

Rice and Lawless (1957) found no evidence for reverse turning in planarians at a choice point following a forced turn. The possible influence of thigmotaxis was eliminated by discarding all worms which followed the wall of the maze after making the forced turn. Of course, this criterion would eliminate all worms which had attempted to alternate their turning direction following the forced turn but before reaching the choice point and, as a consequence, had come into contact with the outside wall.

Dingle (1961a, 1962) has reported that the boxelder bug, *Leptocoris trivittatus*, and several other insects, when forced to make a right-angle turn on an elevated maze, tended to turn in the opposite direction rather than continue straight ahead. In *Leptocoris* the tendency to alternate turning directions increased both when the distance between starting point and forced turn was increased and when the distance between forced turn and choice point was decreased. Dingle suggested

that reverse turning is an adaptive behavior peculiar to insects living on the outer leaves of bushes and branches. He interpreted the response essentially as a manifestation of a forward-going tendency. Like Warden, Jenkins and Warner (1940), Dingle believed that reverse turning was an attempt by the organism to re-establish a "set" which had been created by sensory inflow before encountering the forced turn. In these first experiments with boxelder bugs an open window served as the light source. Dingle performed control experiments in order to eliminate the possibility that phototaxis was the basis for reverse turning. Another possibility not discussed by him was that rather than being a simple phototaxis, the response was a menotaxis, or compass reaction, in which the bug moved at a fixed angle to the light source. This explanation would be compatible with Dingle's analysis of the response. Thus, the "set" established prior to the forced turn would be the bug's orientation relative to the asymmetrical light field. Following the forced turn the bug would temporarily attempt to regain its original orientation or "set" until it assumed its newly imposed one. A concurrent report by Dingle (1961b) that the reverse turning tendency was reduced as the intensity of illumination was reduced indicated that vision indeed was involved in the response.

In a recent paper Dingle (1964a) has reported additional characteristics of the reverse turning tendency in boxelder bugs. The tendency was present to a small but statistically significant extent in blinded bugs. Both normal and blinded bugs exhibited the tendency following forced runs on curved causeways; the degree of reverse turning was increased as the distance run was increased and as the radius of curvature of the causeway was decreased. Dingle was able to quantify the turning tendency by substituting a level platform for the choice point; upon the platform and about the point of emergence from maze pathway onto platform were inscribed arcs divided into  $10^\circ$  sectors. With this apparatus he was able to describe in terms of angular turning tendency the effects both of forced runs on curved causeways and of different starting point to forced turn distances. Also, he indicated that Akre (1962), using mazes similar to his own, had found reverse turning in the red milkweed bug, *Tetraopes tetraophthalmus*.

A different approach to turning reactions has employed the simple T-maze. The turning phenomenon observed in the T-maze is referred to as "spontaneous alternation." In this maze the animal is required to make right-angle turns at the junction of the T on successive trials. On the initial trial the animal may be either permitted a free choice or forced to enter one of the arms. It is then returned to the starting arm, and its choice on the second trial is recorded as a repeat or alternation of the initial choice. White rats, which have been studied most extensively in this maze, display a strong tendency to alternate.

Use of the T-maze eliminates the mechanical components of centrifugal swing, momentum and the subsequent contact response. However, a large body of work, reviewed by Dember and Fowler (1958), has demonstrated that the turning response in such a maze is in fact very complex. When a rat is subjected to a second trial in the same maze it is exposed to the same places and stimuli already encountered on the preceding trial. With the introduction of these variables, it is possible that the rat may alternate its turning with respect to one or a combination of factors. The following are two examples of interpretations which have been suggested at various times for spontaneous alternation. First, alternation may be



the result of a persisting negative reaction to the turning response of the preceding trial, as the proponents of reactive inhibition have suggested. Thus, spontaneous alternation and reverse turning would have the same basis, that is, alternation of turning with respect to an immediately preceding *response*. This theory, however, has been found to be inadequate for explaining a number of aspects of spontaneous alternation. Second, alternation may occur with respect to the *place* and *stimuli* encountered on the preceding run. In line with this second viewpoint, spontaneous alternation has been interpreted in terms of exploratory drive, curiosity, and stimulus satiation, and it is upon these factors that recent psychological research has focused.

A third explanation for spontaneous alternation, which has not been considered by alternation theorists, is that under certain circumstances spontaneous alternation may reflect the forward-going tendency proposed for rats by Dashiell and Bayroff (1931). Alternation on the second trial would result from a persisting tendency to correct for the forced turn experienced on the initial trial. This explanation is similar to reactive inhibition because it states that spontaneous alternation and reverse turning are each manifestations of the same behavioral tendency and both are reactions to the preceding forced response. Although it is now established that psychological factors, such as curiosity, may be important determinants of spontaneous alternation, this third explanation is advanced because it may be significant in maze situations, and in organisms other than white rats, where the psychological factors are not dominant or even involved.

One of the rat experiments possibly germane to the experiments to be described herein is the report of a spatial gradient in alternation tendency (Zeaman and Angell, 1953). Rats were run on a fan-shaped elevated maze consisting of choice alleys located at 90° to the right, 30° to the right, 30° to the left, and 90° to the left of the starting arm. The rats were first forced for either two or ten runs onto one of the 90° alleys and were then given a free-choice trial in which all alleys were open. There was a tendency to choose with increasing frequency the alleys farther removed from the original forced alley. The gradient of responses was more pronounced after ten forced trials than after two forced trials.

Spontaneous alternation has been reported in earthworms. *Lumbricus terrestris* was found to alternate on successive trials in a T-maze (Wayner and Zellner, 1958). When the suprapharyngeal ganglion was removed, the tendency to alternate was reduced and in some worms was replaced by a tendency to repeat the preceding choice. In a recent review Jacobson (1963) describes the following additional unpublished experiments on alternation in annelids. Arbit and McLean (1959) employed the same technique used with rats by Zeaman and Angell (1953) but failed to find a spatial gradient in alternation tendency in *Lumbricus*. Fraser (1958) found that the earthworm, *Allolobophora terrestris longa*, alternated turns in a T-maze significantly more often than chance expectation, and the earthworm, *Lumbricus rubellus*, alternated significantly less often. Kasper (1961) has reported that an earthworm can exhibit reverse turning in a maze pathway consisting of a forced turn followed by a free choice point.

Iwahara (1956) found no evidence for spontaneous alternation in cockroaches run in a Y-maze with inter-trial intervals of either 20 or 120 seconds.

From the foregoing review it is evident that in a number of experiments there

was failure to control for such well established behavioral responses as thigmotaxis, phototaxis, menotaxis, and in some cases, possibly, the following of chemical trails. Perhaps part of the reason for this failure is that many authors have regarded reverse turning and spontaneous alternation primarily as maze phenomena and have not recognized their possible biological significance as important components of animal orientation reactions. The further possibility that reverse turning represents a kinesthetic response was suggested more than twenty years ago, and good evidence for it has been provided by recent experiments. It is notable that two reviewers of animal orientation, Lindauer (1963) and Jander (1963), have considered the experiments on reverse turning in mealworms (Grosslight and Harrison, 1961) and boxelder bugs (Dingle, 1961a) as examples of kinesthetic orientation. For boxelder bugs it has been pointed out that more convincing evidence for a kinesthetic contribution was actually provided by later experiments in which visual cues were eliminated by blinding the bugs (Dingle, 1964a).

The following experiments demonstrate a reverse turning tendency in another group, the Diplopoda. It has been possible to show that a millipede species is capable of precise quantitative compensation for forced turning through a large range of angles from an initial path.

#### MATERIALS AND METHODS

Tropical millipedes, *Trigoniulus lumbricinus* (Gerstaecker 1873), were ideal for experimental use. They were quite tractable, crawled about actively when handled, and did not appear to fatigue during an experiment. Generally they were collected on the day of the experiment from leaf litter beneath a breadfruit tree in the garden of the Museu Goeldi in Belém, Brazil, where all experiments were performed. At least one-half hour before being tested, each animal was placed in the dimly illuminated orientation chamber for a period of light adaptation. The animals used were approximately 4 mm. in width and ranged from 43 to 54 mm. in length.

The object of experimental manipulation was to force the millipede to turn from an initial path through a specified angle and to measure any subsequent orientative response to the forced turning. This was accomplished by causing the animal to crawl through a narrow corridor, in the middle of which was an abrupt turn. When the animal emerged from the corridor, its amount of right or left turning was measured in terms of degrees of angular deviation from a straight-ahead path. One of the corridors used is illustrated in Figure 1. A series of such corridors was constructed of smooth wooden blocks glued to sheets of graph paper. The dimensions of all corridors were the same: total length, 14 cm.; height, 7 mm.; and width, 6 mm. Only the angle of the forced turn was varied. For measuring the angle of emergence an arc was circumscribed on the graph paper at a distance of 6.35 cm. from the exit and divided into 5° sectors. The animal's orientation was recorded as the sector in which its head first reached the arc. A transparent glass plate served to cover the corridor. Dingle (1964a) has described a similar method for quantifying the reverse turning tendency in boxelder bugs.

The length of each of the three components of the experimental path, corridor entrance to turn (7 cm.), turn to corridor exit (7 cm.), and corridor exit to arc (6.35 cm.), exceeded the length of any animal used. Consequently, each animal was

required to enter the corridor completely before encountering the turn, to straighten out completely between forced turn and exit, and to crawl a distance greater than its length from corridor exit to arc. Also, the corridor was sufficiently wide to permit the animals to emerge without touching either wall.

The visual environment of the millipedes was controlled by placing the corridor in use within a large chamber, which was 49 cm. high, 79 cm. wide, and 62 cm. long. An opening 30 cm. wide in the back of the chamber behind the corridor permitted observation and access for handling the animals. The interior of the chamber was painted mat black. It was dimly illuminated through a small hole, in

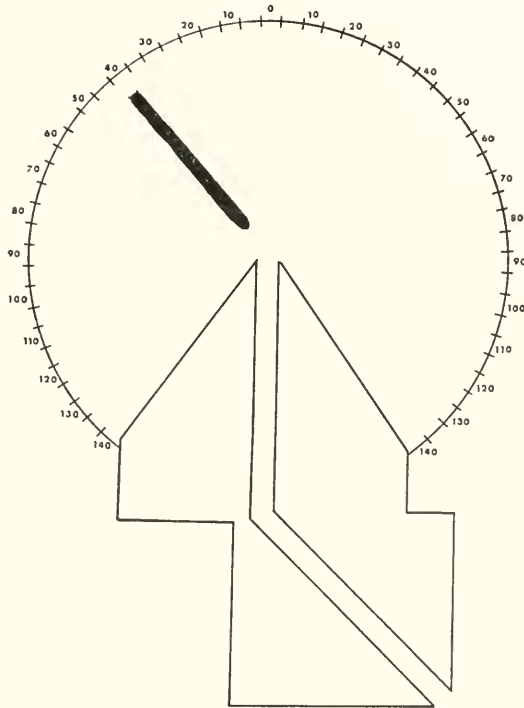


FIGURE 1. Orientation apparatus for quantifying reverse turning in millipedes. A millipede is depicted as responding to a  $45^\circ$  forced turn.

the center of the top of the chamber, which was 1.5 cm. in diameter and covered with a white diffusing transmitter. The light source was a 25-watt frosted incandescent bulb centered 1.5 cm. above the small opening. During all experiments the exit of the corridor was directed southward and was centered directly under the light spot. Thus, at the instant of the animal's emergence from the corridor, the overhead light source itself could not serve as a horizontal directional reference cue. The experiments were always performed in a darkened room.

The experiments consisted of measuring the response of individuals to each of a series of angular turns. Two different series were used. The first, which will be referred to as Series I, consisted of forced right-hand turns and comprised the

following angles:  $0^\circ$  (no turn),  $15^\circ$ ,  $30^\circ$ ,  $45^\circ$ ,  $60^\circ$ ,  $75^\circ$ ,  $90^\circ$ ,  $105^\circ$ , and  $120^\circ$ . Between Nov. 15 and Dec. 2, 1962, 45 animals were run through this series at various times of day from 7 AM to 11 PM. The average time required to complete an entire experimental series with a single animal was 29 minutes. The second series, designated Series II, comprised the following seven angles, ranging from  $30^\circ$  to the right to  $30^\circ$  to the left:  $30^\circ$ ,  $20^\circ$ ,  $10^\circ$  to the right;  $0^\circ$ ;  $10^\circ$ ,  $20^\circ$ , and  $30^\circ$  to the left. Between Nov. 29 and Dec. 11, 1962, 50 animals were run through this series. The average time required for a single animal was 17 minutes. In both series the response to each angle for each animal was taken as the average of five consecutive trials.

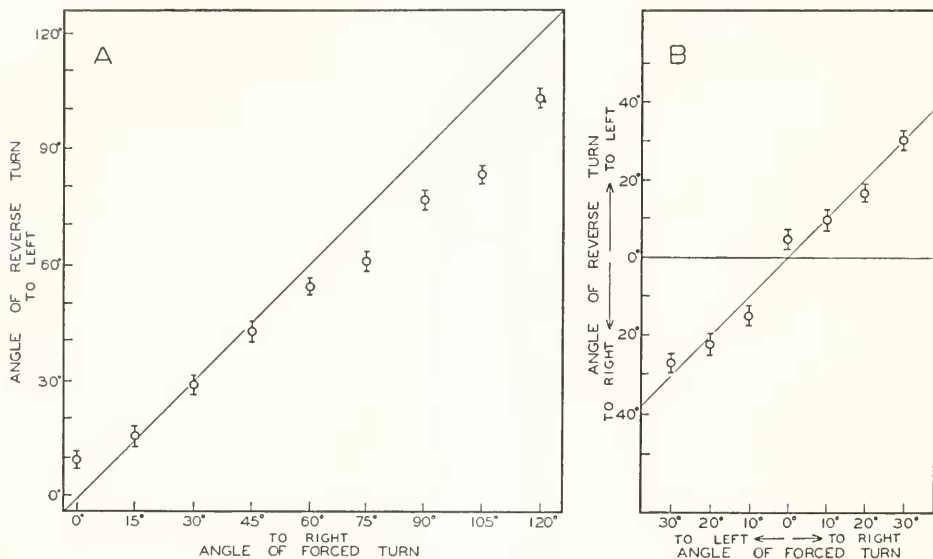


FIGURE 2. A. Mean angular turning response of millipedes to the forced angles of Series I. Standard errors of the mean are indicated. Diagonal line has a slope of 1. B. Same as A, but for Series II.

The procedure was the following. First, the animal's longitudinal axis was carefully aligned in front of the corridor entrance. The animal then was permitted to crawl through the corridor and across the arc. The orientation was recorded, and the animal was brushed lightly back to the corridor entrance where it was realigned and permitted to run again. When five runs had been completed the corridor was replaced by one with a different forced angle. The sequence in which the angles were presented to each animal was a random one, having been determined in advance by lottery.

Statistical formulas used in the analysis of data were those given by Walker and Lev (1953).

## RESULTS

Results of the two experimental series are presented in Figure 2 and Table I. It is obvious that upon emerging from the corridor the millipedes turned on the



average at an angle which was opposite, and in most cases approximately equal, to the angle of the forced turn. Clearly, the millipedes were alternating their turning quantitatively in a precise manner. That the precision of the response was essentially uniform over a wide range of forced angles was indicated by the simi-

TABLE I  
*Means, variances and differences between means of responses  
by millipedes to angular forced turns*

Series I				
Angle of forced turn	Mean (degrees)	Variance (degrees)	Difference between adjacent means (degrees)	Probability (two-tailed <i>t</i> test of differences)
0°	9.49	216.21		
15° R	15.51	279.53	> 6.02	.06
30° R	28.84	259.23	>13.33	<.001
45° R	42.53	295.85	>13.69	<.001
60° R	54.13	232.06	>11.60	<.001
75° R	61.00	301.11	> 6.87	<.005
90° R	76.90	267.01	>15.90	<.001
105° R	83.43	280.16	> 6.53	<.005
120° R	103.63	326.40	>20.20	<.001
Series II				
30° R	30.48	302.95		
20° R	16.66	200.56	>13.82	<.001
10° R	9.84	346.74	> 6.82	<.005
0°	4.56	293.90	> 5.28	.02
10° L	-15.56	296.82	>20.12	<.001
20° L	-22.78	394.71	> 7.22	<.02
30° L	-27.68	251.24	> 4.90	.07

larity of variances for all angles (Table I). Comparison of mean values for the two independent series, I and II, revealed that the means for responses to the same angle, 30° to the right, were similar. Means for responses to intermediate angles in Series II, 20° and 10° right, were intermediate, although each of these two angles was not significantly different from means of responses to both of the forced

angles in Series I which bracketed each of them. In both series the  $0^\circ$  response was asymmetrical, being twice as far to the left in Series I as in Series II.

In Series I the relationship between stimulus intensity and response intensity was not linear throughout the entire range of forced angles. From  $15^\circ$  to about  $60^\circ$  the angle of emergence was equal to the angle of the forced turn; above  $60^\circ$  the average angular turning upon emergence for all experiments was significantly less than the angle of the forced turn. For Series II the average values for all seven angles fell close to the regression line described by the equation  $Y = X$ , which indicates equivalence of stimulus and response. The computed least squares regression equation,  $Y = 0.6^\circ + 0.98X$ , is scarcely distinguishable from this theoretical relationship.

TABLE II  
*Analysis of variance for comparing the turning responses of individuals to a series of angular forced turns*

Series I				
Source of variation	Sum of squares	Degrees of freedom	Mean square	F
Total group	473,799.2	404		
Between means of individuals	43,704.1	44	993.28	5.427*
Between means of forced turns	365,666.8	8	45,708.35	249.731*
Error	64,428.3	352	183.03	
Series II				
Total group	238,664.6	349		
Between means of individuals	49,733.7	49	1,015.0	6.416*
Between means of forced turns	142,428.1	6	23,738.0	150.051*
Error	46,502.8	294	158.2	

\*  $P < 0.001$ .

Measurements for all angles in a series were made on every individual. Therefore, it was possible to test with an analysis of variance for the significance of differences both in response to forced angles and in the response of individuals. In each series both responses to forced turns, as was evident from inspection, and the individual responses were highly significantly different from chance expectation (Table II).

A more detailed examination of the differences in response to forced angles was made by testing the significance of the difference between the means of responses to adjacent forced angles in each series. Comparisons were made with a  $t$  test in which the data were treated as non-independent samples in order to eliminate variation due to individual differences. For each pair of forced angles the significance of the mean of a population of differences between two measures on each individual

was tested. Hence, the probabilities given in Table I for differences between means were not based on the variances listed in the neighboring column. In Series I, where 15° intervals separated adjacent forced turns, the means for all adjacent angles were highly significantly different with the exception of the difference between 15° and the asymmetrical 0° response (Table I). In Series II, where 10° intervals separated the forced angles, the probabilities were not so uniformly high as in Series I. Nevertheless, it appeared that on a statistical basis the millipedes were capable of distinguishing 10° differences in forced angles, even when the forced angle itself was only slightly larger than 0°.

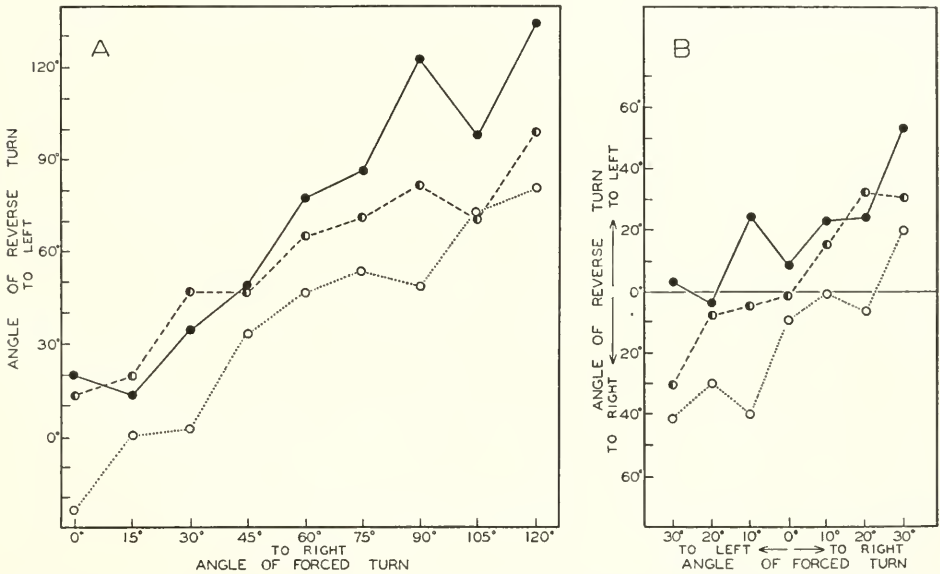


FIGURE 3. A. Mean angular turning response to forced angles of Series I for three millipedes selected from six run on Nov. 19, 1962. B. Same as A, for three millipedes selected from seven run on Dec. 1, 1962, in Series II.

The extent of the highly significant individual variation is indicated in Figure 3 by selected examples from each of the experimental series. Figure 3A represents the values for three individuals out of six which were run through Series I on Nov. 19. Figure 3B represents three out of seven individuals run through Series II on Dec. 1. There is a slight suggestion that the regression equation describing responses to a series of forced angles may differ in slope as well as intercept among individuals. The range of variation illustrated here is typical of that encountered among individuals on other days.

There is an additional large component of the variance in Series I which was not accounted for in the preceding analysis of variance. This variance is associated with the order in which the nine different forced angles were presented within individual series. Since this order was shuffled in every case, each of the forced angles happened by chance to be presented at least one in each of the positions

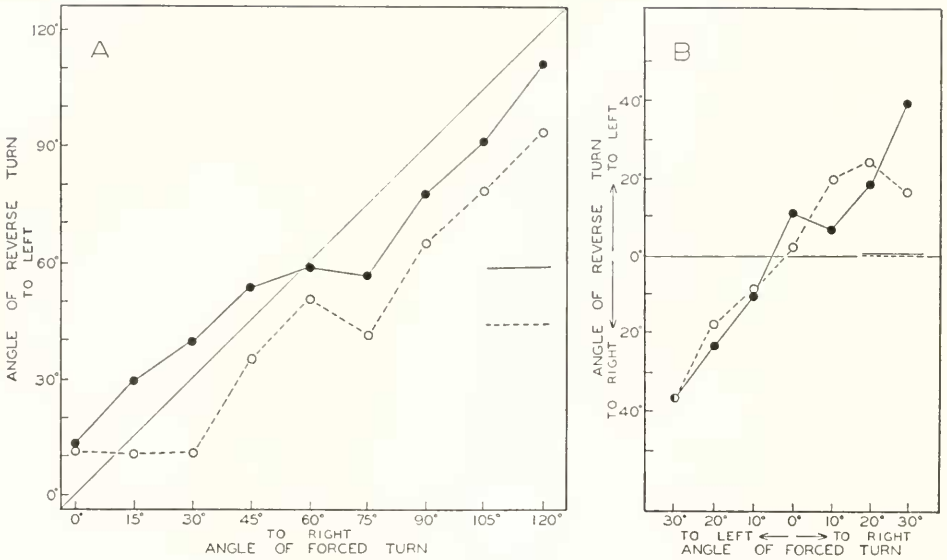


FIGURE 4. A. Mean angular turning response for all millipedes in Series I run first in sequence (open circles, dashed line) and ninth in sequence (closed circles, solid line). Mean values for each of the two series are indicated by horizontal lines to the right in the figure. Diagonal line has a slope of 1. B. Same as A, but for Series II.

from first through ninth. There was, however, one exception to this statement. The 15° forced turn never occurred fifth in any series. For the analysis to be presented in Figure 5A a value was interpolated for the response to this forced angle by taking the average of the mean responses to the 0° and 30° forced turns occurring fifth in sequence. The extent to which the response was affected by sequence position is shown in Figure 4A where the mean value for each forced

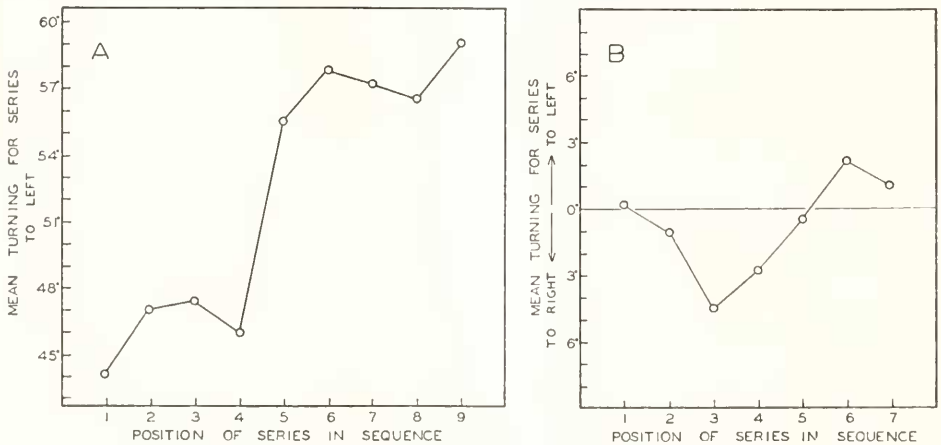


FIGURE 5. A. Mean angular turning for series occurring in each position in sequence in Series I. B. Same as A, but for Series II.

angle presented first in sequence is compared with the mean value when each angle was presented ninth, or last, in sequence. For all forced angles the response to the angle when it occurred ninth in order was greater than, or to the left of, the response to the same angle when it occurred first. The difference was largest at  $15^\circ$  and  $30^\circ$ , where it appeared that the animals did not distinguish among forced turns of  $0^\circ$ ,  $15^\circ$ , and  $30^\circ$  on the first trial. To the right in Figure 4A two lines indicate the average difference between means for all nine angular forced turns in the two positions, first and ninth. The difference is  $15^\circ$ . The progressive character of the increase in turning tendency is shown in Figure 5A where the means for

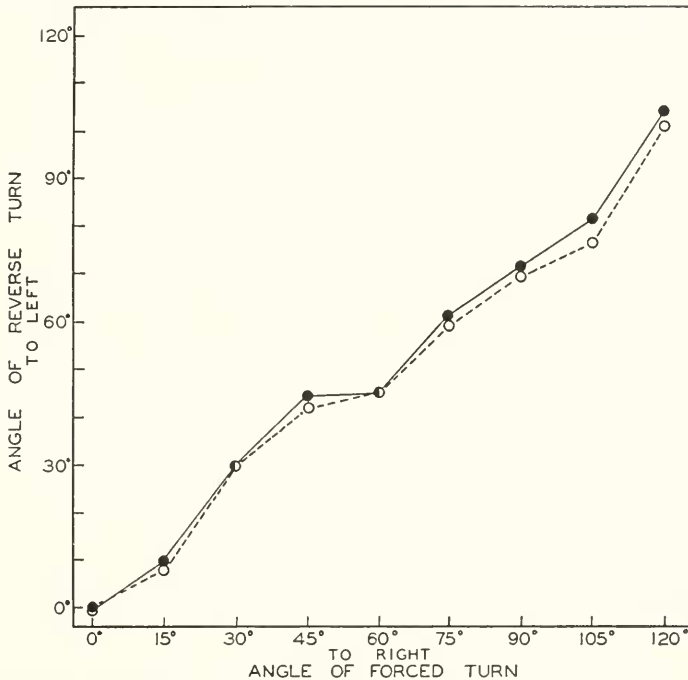


FIGURE 6. Mean angular turning response to forced angles for the last seven millipedes in Series I measured at two distances from the corridor exit, 6.35 cm. (open circles) and 10.2 cm. (closed circles).

the angles of Series I occurring in each of the orders of first through ninth are graphed. These averages include the responses to the  $0^\circ$  corridor, although for this one angle there was no obvious trend toward displacement of the response to the left as one progressed through the nine orders of sequence. Values initially were low, increased rapidly between fourth and fifth positions and tended to level off during later positions.

When the data for Series II were subjected to the same analysis, no consistent differences were found (Figs. 4B and 5B). It is evident, however, that animals were responding on the first trial to forced angles of  $30^\circ$  and less.

As it emerged from the corridor the millipede oriented sharply and crawled off



on a fairly straight course; the animal showed no obvious tendency to circle in either direction. In order to determine the directness and stability of the orientation, a second, concentric arc was traced on the orienting surface at a distance of 10.2 cm. from the exit. Comparison of the orientation angles of each millipede at two distances from the corridor exit, 6.35 and 10.2 cm., would disclose any tendency in the animal for continued turning after it had crossed the first arc. This additional measurement was made on the last seven millipedes run in Series I. The results are shown in Figure 6. It is evident that essentially all turning had been completed before the animal reached the first arc at 6.35 cm. from the exit. Dingle (1964a) has reported a slight but not statistically significant tendency for boxelder bugs to continue turning after making the initial reverse turning response. In millipedes the tendency to continue turning is much less than it is in boxelder bugs for turning responses of comparable size,  $55^\circ$  and less.

### DISCUSSION

In this study the millipede, *Trigoniulus lumbricinus*, was found capable of executing a reverse turn which was, on the average, precisely related to the angle of a preceding forced turn. The animal, therefore, appeared to possess an accurate angle sense. Furthermore, since the reverse turning response was delayed while the animal was forced to straighten out completely in a narrow corridor, the animal necessarily possessed a memory for the forced angle.

With the type of orientative apparatus used, the possibility that thigmotaxis, or contact response, was involved in the reverse turning reaction was unavoidable. However, it is doubtful that a simple thigmotaxis could account for quantitative compensatory turning. The  $0^\circ$  corridor served as a control for the thigmotactic component of reverse turning. In both experimental series the mean response to this corridor was closer to  $0^\circ$  than for any other forced angle. Of course, a mean response of  $0^\circ$  could result if the millipedes turned strongly with equal frequency to right and left. On the other hand, for the responses to the  $0^\circ$  corridor the variance, which would indicate the degree of strong right and left turning, was not significantly larger than the variances for other forced turns. Thus, a large thigmotactic response was not expressed on runs through the  $0^\circ$  corridor. Furthermore, it is presumable that thigmotaxis should influence responses for all forced turns, equally, since all such turns would tend, in the narrow corridor, to bring every animal into contact with the outside wall of the turn. It was observed that sometimes after making a forced turn the millipede would not follow the outside wall of the turn. Instead, it would remain close to the inside wall, but would constantly tap the outside wall with its antenna. Upon emergence from the corridor the animal would alternate its turning in the expected direction.

The tendency of the animal to follow by means of olfactory cues the trail of its own or another animal's earlier run did not appear to be an important factor. This is indicated, for instance, by the highly significant individual differences presented in Table II and Figure 3. Further evidence was provided by a brief preliminary experiment in which the same sheet of paper was used as the orienting surface for a series of forced turns. Although each animal had the opportunity of following its own or another millipede's trail for the same or different forced angles, the

results were the same as for the experiments reported here, namely, significant differences among individuals and angles.

The effect upon reverse turning of varying the angle of the forced turn has been examined in boxelder bugs by Dingle (1964a). He measured the response to a range of forced angles by determining at the choice point the percentage of bugs that made a right-angle turn in the opposite direction rather than continuing straight ahead. He found that the percentages exhibiting reverse turning after  $0^\circ$ ,  $30^\circ$ ,  $45^\circ$ , and  $60^\circ$  forced turns were approximately the same, ranging from 19% to 23%, while the percentages after  $75^\circ$  and  $90^\circ$  forced turns were significantly greater, being 44% and 47%, respectively. He concluded (p. 119), "Rather than a steady increment in correcting as the amount of turn increased, there was a certain critical angle at which a marked and significant increase occurred." On this point the behavior of boxelder bugs apparently contrasts with that of the millipedes, which showed significantly different responses to all the angles used by Dingle. On the other hand, it is possible that the boxelder bug, too, possesses a capacity to respond to smaller forced angles but did not manifest this capacity under the conditions of Dingle's experiments. Two possible explanations for the apparent absence of this capacity are presented here. First, it was suggested in Series I (Fig. 3A) that millipedes did not respond to forced angles of  $15^\circ$  and  $30^\circ$  when these were presented first in sequence. Only after several forced runs through the corridor did a response to these smaller forced angles become evident. Possibly boxelder bugs require several forced runs before responding to forced angles smaller than  $75^\circ$ . If Dingle gave the bugs only a single trial, they may not have been in a satisfactory physiological state for evincing a response to small angles. Second, the apparent absence of correcting for small forced angles may be due to the fact that the response was measured with a dichotomous scale, in which the bug was forced to choose between the alternatives of making a  $90^\circ$ -reverse turn or no turn at all. Although boxelder bugs may be capable of precise compensation for forced angles between  $0^\circ$  and  $60^\circ$ , even on the first run, this gradient of response could be masked if the correcting tendency were not strong enough to produce right-angle turning, and so led to a predominant choice of the straight path. Supporting this possibility would be the fact that no critical angle was found when Dingle used a continuous scale, that is, measurement of angular turning tendency over a  $180^\circ$  arc, to quantify the response of bugs to various curved causeways.

It is of interest to compare the reverse turning tendency reported here with the homostrophic reflex described for millipedes by Crozier and Moore (1923). In this latter reaction a lateral displacement of the tail brings about a compensatory turning of the head so that its orientation is parallel and in the opposite direction to that of the tail. Jander (1963) has distinguished in any taxis mechanism two basic components, an afferent angle sensing and directing one and an efferent coordinating one. It may be that the two component mechanisms are similar in reverse turning and the homostrophic reflex, but the latter does not require a memory for the angle of bending of the body as does the reverse turning reaction. Possibly, however, Crozier and Moore were observing a memory for angle when they reported (1923, p. 600), "The fact that the reflex may be somewhat delayed increases the appearance of 'intelligent' pursuit of a straight path."

Precautions were taken to reduce the significance of the light source as an

external orientative reference cue for menotactic, or compass, reactions. This, plus the similarity of the response to the homostrophic reflex, which is dependent upon proprioceptive cues, suggests that the reverse turning reported here is basically a kinesthetic response. Such an internally controlled kinesthetic response would, of course, nicely supplement in a functional manner any externally controlled responses, including menotaxis and astrotaxis, which serve to maintain the organism on a directed course.

It was not determined if any visual input at all was necessary for quantitative reverse turning in *Trigoniulus*. Apparently it plays a role in the boxelder bug, since blinding the bug diminishes its turning tendency (Dingle, 1964a). For a tropical millipede which normally inhabits the gloomy floor of the rain forest it is conceivable that photoreception would be relatively less important than proprioception for the response.

It should be noted, however, that the experiments as performed did not eliminate the possibility that pervasive extra-maze factors served as spatial references for a compass reaction. The recent demonstrations that animal orientation may be affected by very weak magnetic, electrostatic, and gamma radiation fields (Brown, 1962a, 1962b, 1963; Schneider, 1963) indicate that at least such a possibility should be considered.

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#### SUMMARY

1. The millipede, *Trigoniulus lumbricinus*, when forced to crawl through a corridor containing an abrupt turn, tended to turn upon emergence from the corridor at an angle which was opposite and approximately equal to the angle of the forced turn.

2. The response held for forced angles ranging in size up to at least 120°. For forced turns greater than 60° the amount of reverse turning was less than the amount of forced turning. On a statistical basis the millipedes appeared to be capable of distinguishing 10° differences in the angle of the forced turn.

3. There were highly significant differences in the response patterns of individual millipedes to a graded series of forced angles.

4. The turning tendency was significantly increased following a series of turns in the same direction.

5. The precision and sensitivity of the reverse turning response suggest that it is an important orientation reaction for millipedes. A brief survey of the literature on certain maze-running phenomena, reverse turning and spontaneous

alternation, further suggests that the response is a type of kinesthetic orientation which may prove to be widespread in animals.

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